

05470667-1330-29904460

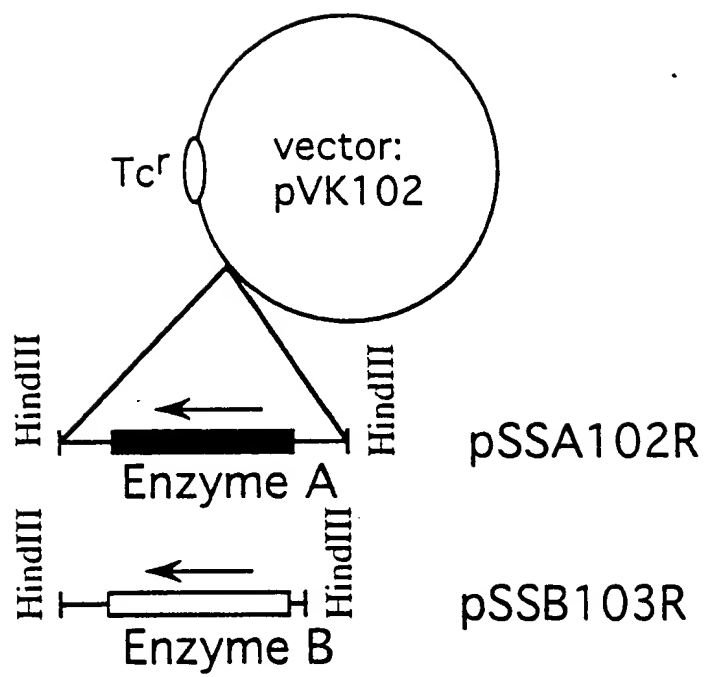


Fig. 1.

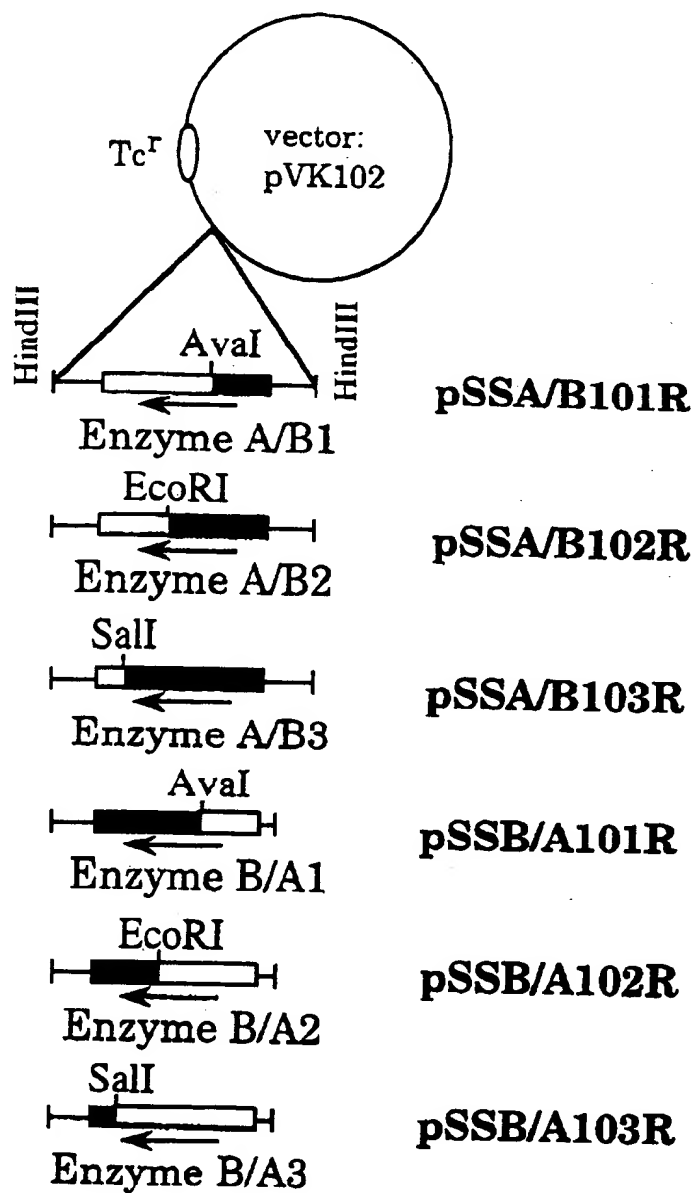
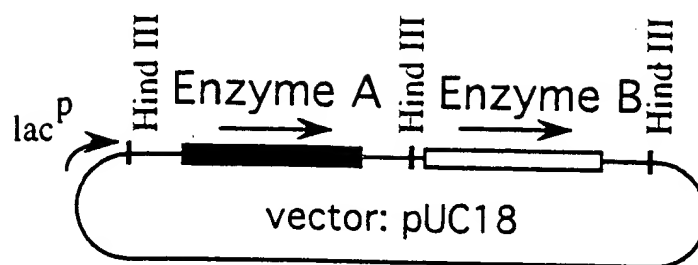


Fig. 2.

pSSAB201



pSSBA201

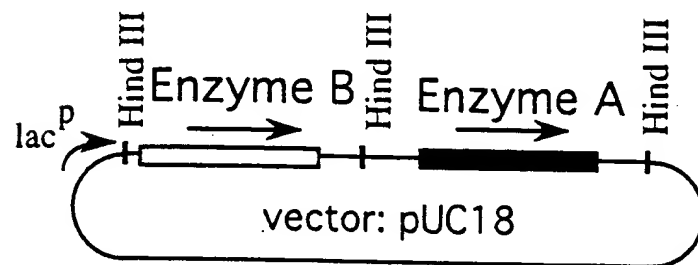
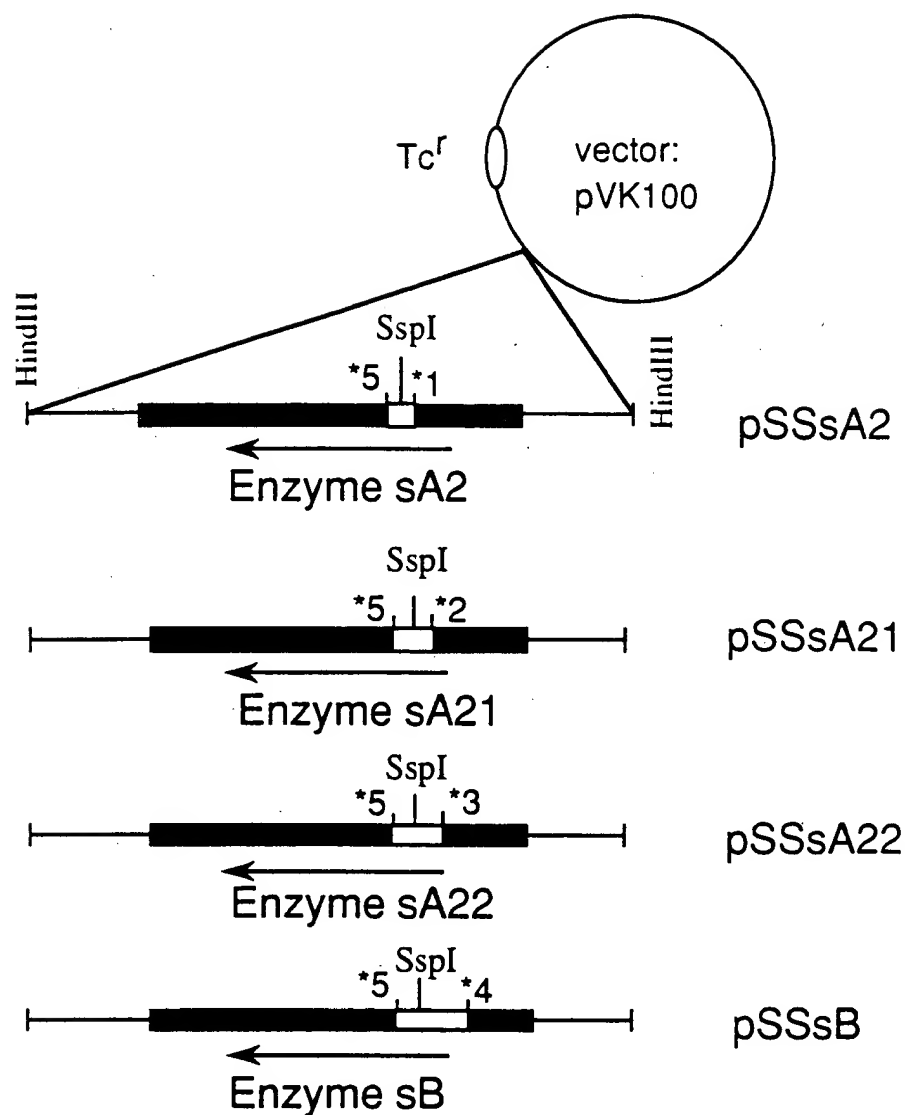


Fig. 3.



#### Recombination site

- \*1 : amino acid residue No. 135 of mature Enzyme A
- \*2 : amino acid residue No. 128 of mature Enzyme A
- \*3 : amino acid residue No. 125 of mature Enzyme A
- \*4 : amino acid residue No. 95 of mature Enzyme A
- \*5 : amino acid residue No. 180 of mature Enzyme B,  
which nucleotide sequence of Aval site encodes

*what  
rejection*

Fig. 4.

Enzyme A 1 : QVTPVTDELL ANPPAGEWIS YGQNQENYRH SPLTQITTEN VGQLQLVWAR GMQPGKVQVT  
 Enzyme B 1 : QVTPITDELL ANPPAGEWIN YGRNQENYRH SPLTQITADN VGQLQLVWAR GMEAGAVQVT

61 : PLIHDGVMYL ANPGDVIQAI DAKTGDLINE HRRQLPNIAT LNSFGEPTRG MALYGTVNYF  
 61 : PMIHDGVMYL ANPGDVIQAL DAQTGDLINE HRRQLPAVAT LNAQGDRKRG VALYGTSLYF

121 : VSWDNHLVAL DTATGQVTFD VDRGQGED-M VSNSSGPIVA NGVIVAGSTC QYSPFGCFVS  
 121 : SSWDNHLIAL DMETGQVVFD VERGSGEDGL TSNTTGPIVA NGVIVAGSTC QYSPYGCFFS

180 : GHDSATGEEL WRNYFIPRAG EEGDETWGND YEARWMTGAW GQITYDPVTN LVHYGSTAVG  
 181 : GHDSATGEEL WRNHFIQPG EEGDETWGND FEARWMTGVW GQITYDPVTN LVFYGSTGVG

240 : PASETQRGTP GGTLYGTNTR FAVRPDTGEI VWRHQTLPRD NWDQECTFEM MVTNVDVQPS  
 241 : PASETQRGTP GGTLYGTNTR FAVRPDTGEI VWRHQTLPRD NWDQECTFEM MVANVDVQPS

#### EcoRI

300 : TEMEGLQSIN PNAATGERRV LTGVPCKTGT MWQFDAETGE FLWARDTNYQ NMIESIDENG  
 301 : AEMEGLRAIN PNAATGERRV LTGAPCKTGT MWSFDAASGE FLWARDTNYT NMIASIDETG

360 : IVTVNEDAIL KELDVEYDVC PTFLLGGRDWP SAALNPDSGI YFIPLNNVCY DMMAVDQEFT  
 361 : LVTVNEDAVL KELDVEYDVC PTFLLGGRDWS SAALNPDTGI YFLPLNNACY DIMAVDQEFS

#### Sall

420 : SMDVYNTSNV TKLPPGKDMI GRIDAIDIST GRTLWSVERA AANYSPVLST GGGVLFNGGT  
 421 : ALDVYNTSAT AKLAPGFENM GRIDAIDIST GRTLWSAERP AANYSPVLST AGGVVFNGGT

480 : DRYFRALSQE TGETLWQTRL ATVASGQAIS YEVDGMQYVA IAGGGVSYGS GLNSALAGER  
 481 : DRYFRALSQE TGETLWQARL ATVATGQAIS YELDGVQYIA IGAGGLTYGT QLNAPLA-EA

540 : VDSTAIGNAV YVFALPQ  
 540 : IDSTSVGNAI YVFALPQ

\* : Nucleotide sequences encoding these regions are the restriction sites for Aval, EcoRI, and Sall which were used for constructing chimera genes shown in Fig. 2.

Fig. 5.

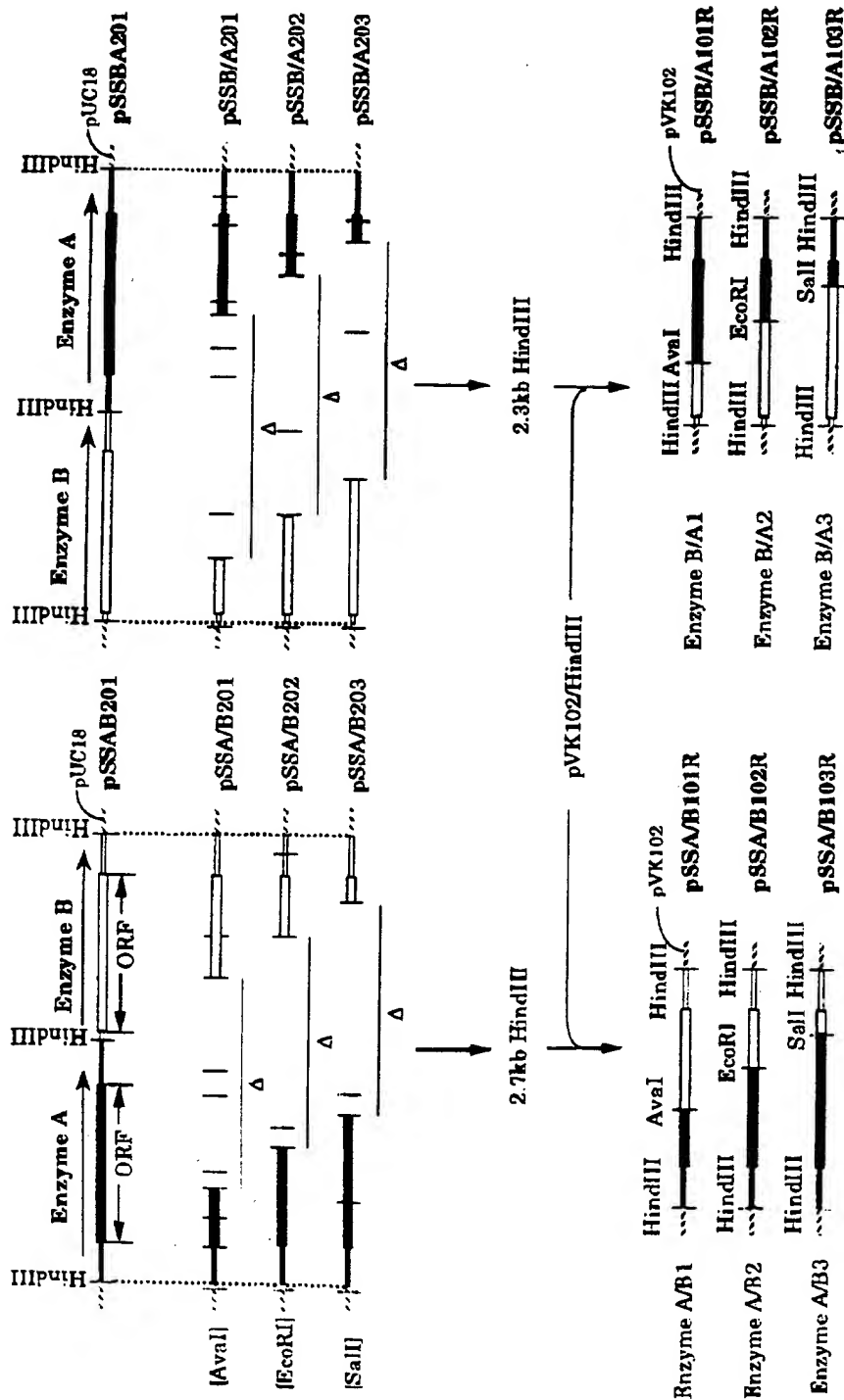
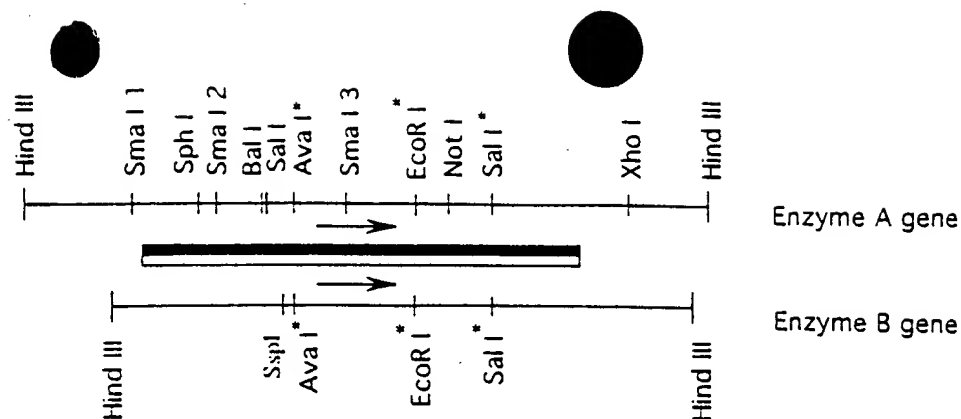
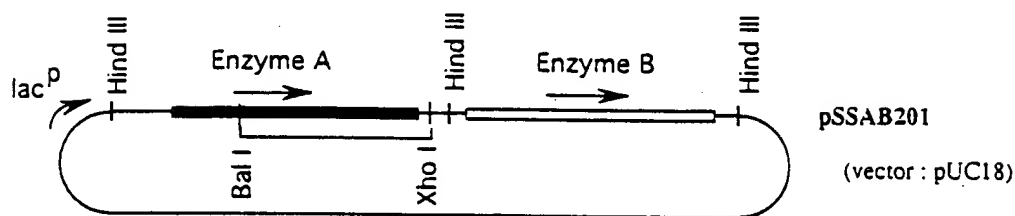


Fig. 6.

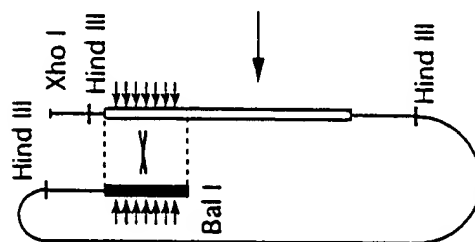


\*: Aval, EcoRI, Sall sites used for constructing chimera genes shown in Figs. 2 and 6.

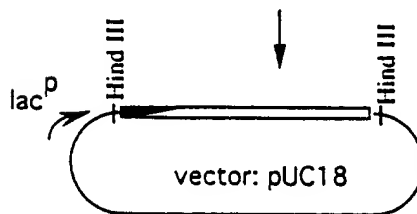
Fig. 7.



Linearization with XhoI and BalI



Transform *E. coli* JM101 (*rec A*<sup>+</sup>)



Various kinds of chimera genes can be obtained.

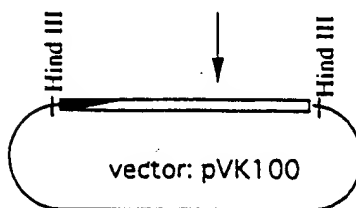


Fig. 8.

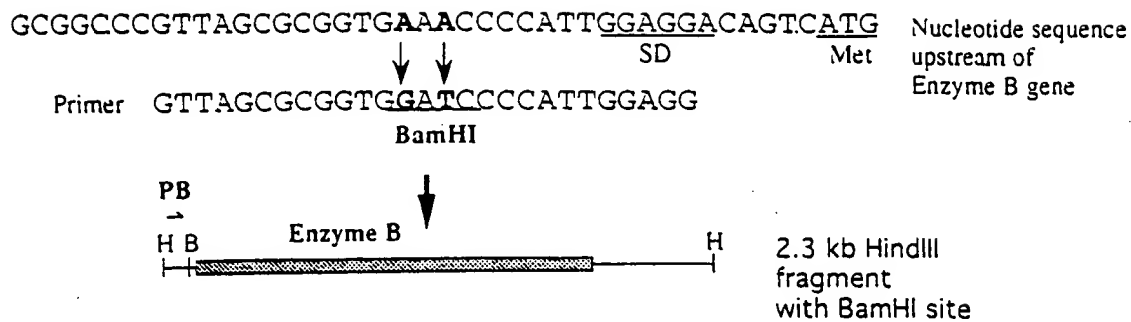


Fig. 9.

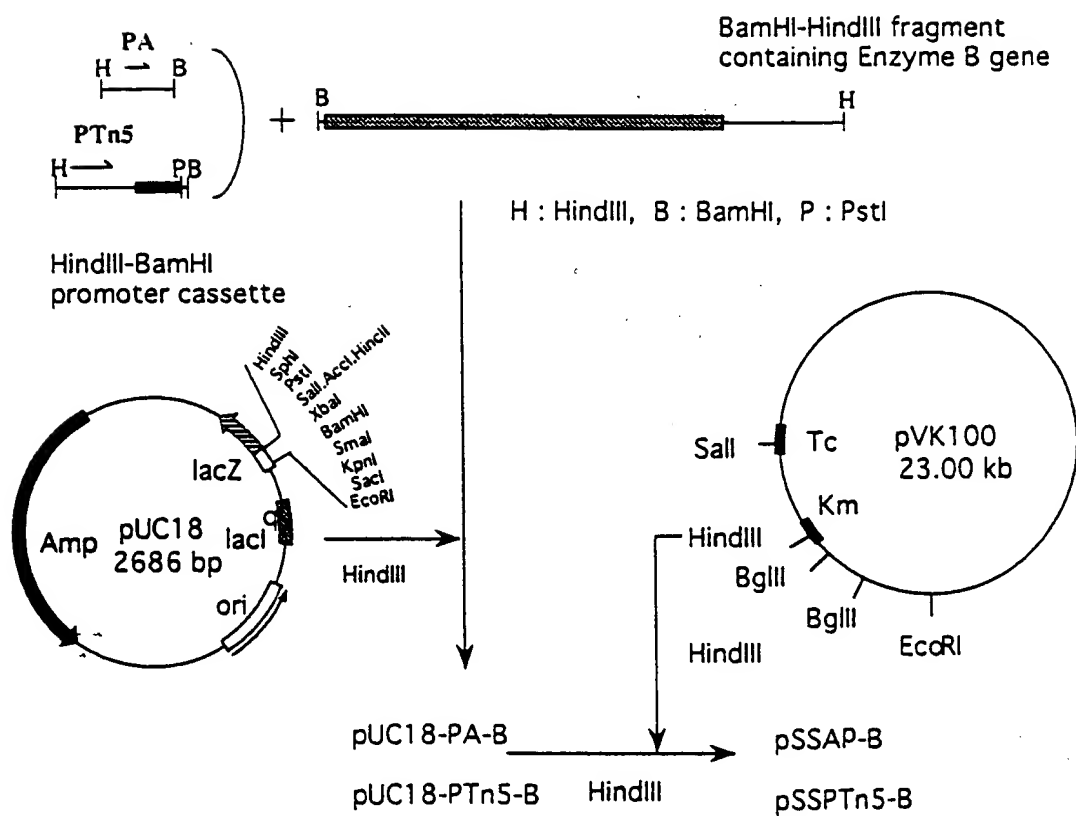
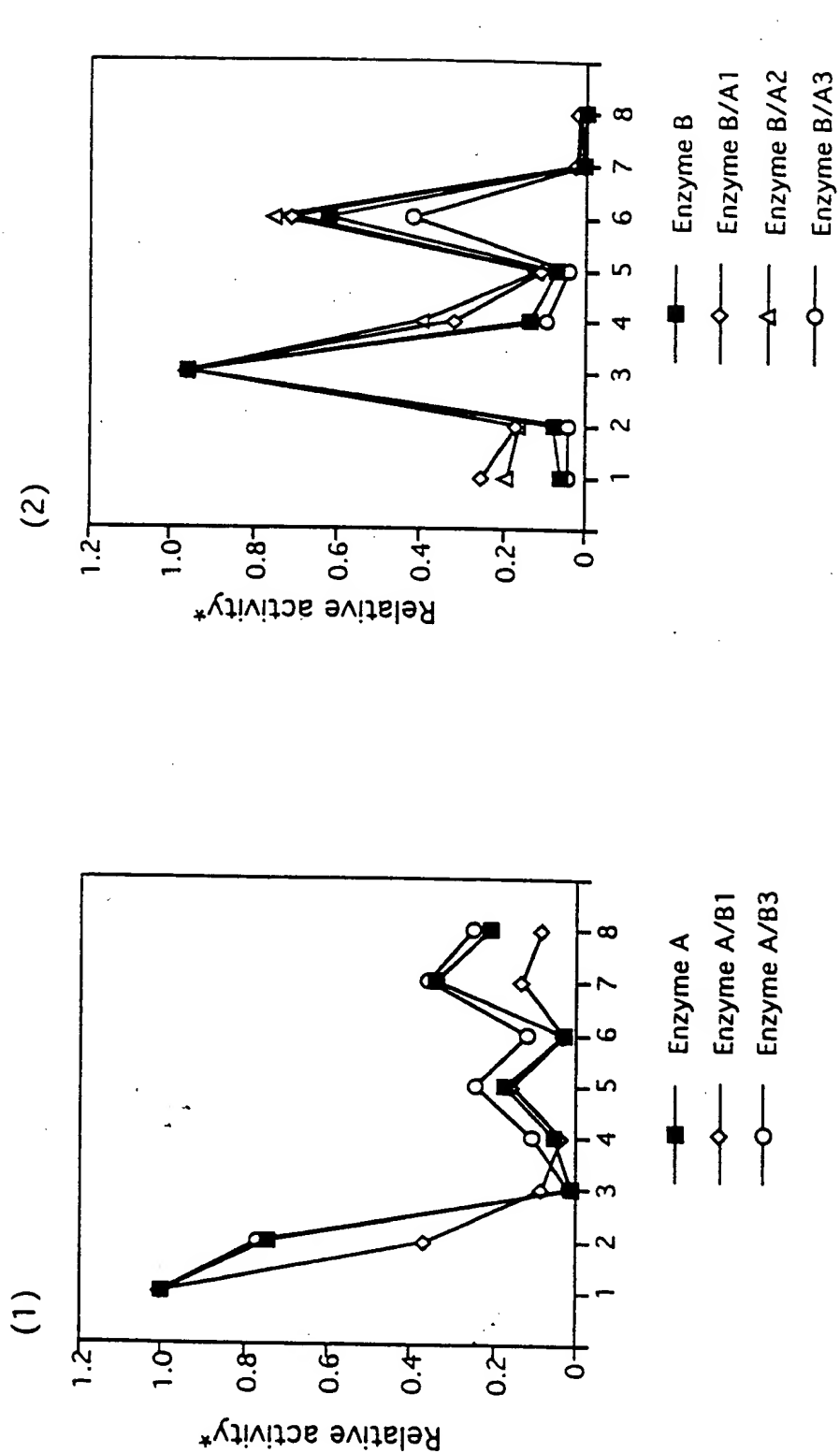


Fig. 10.





1. n-Propanol, 2. Isopropanol, 3. D-Glucose, 4. L-Sorbose  
 5. D-Sorbitol, 6. D-Mannitol, 7. L-Sorbose, 8. D-Fructose

\*Enzyme activity was normalized relative to activity for n-propanol (1), or D-glucose (2). Enzyme A/B2 was excepted because of its low expression in *P. putida*.

Fig. 11.